of immunotherapy treatment. B7-H1 antibody administration significantly improved long-term survival after immunotherapy (40% vs 0% for controls), indicating that the B7-H1/PD-1 pathway inhibits development of myeloma-specific immunity. We are addressing that possibility that other immune suppressive mechanisms can be targeted to further improve immunotherapy efficacy, and several candidates have been identified including $CD4^{+}Foxp3^{+}$ regulatory T cells. These results support the use of B7-H1/PD-1 blocking strategies to increase tumor immunity in myeloma patients.

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BINARY CONTROL OF TUMOR-SPECIFIC CYTOTOXIC T LYMPHOCYTES BY TRANSGENIC IL7 AND IL7 RECEPTOR EXPRESSION

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While adoptive transfer of cytotoxic T lymphocytes (CTLs) engrafted with chimeric antigen receptors (CARs) has produced objective clinical responses in vivo, the infused cells usually fail to persist long-term, limiting benefit. We have recently demonstrated that CTL persistence can be improved by engineering cells to express the IL7 receptor alpha chain (IL7Ra) which is physiologically absent on CTLs, followed by infusion of IL7 cytokine. However, this approach requires access to clinical grade cytokine, and biodistribution of cytokine at tumor sites may be insufficient. To circumvent these problems we have prepared two CTL products; one expressing a tumor-specific CAR in combination with IL7Ra (product #1), and a second engineered to co-express the same CAR and produce IL7 cytokine (product #2). In this way both products have anti-tumor activity, mediated through the CAR, while cytokine produced from CTL#2 should support the survival and persistence of CTL#1 expressing the IL7Ra. A binary system such as this should be intrinsically safer than incorporating a positive feedback loop of both cytokine and receptor in a single cell.

As proof of principal we used the SFG-CAR that targets the kappa light chain, expressed on B cell malignancies. We made two retroviral vectors, SFG-CAR/IL7Ra-GFP (#1) and SFG-CAR/IL7cyto-mOrange (#2) and transduced EBV-CTL from 3 donors with each vector. FACS analysis indicated that all the transgenes were expressed at approx. equivalent levels; CTL#1 (CAR, IL7Ra and GFP; 58%±15, 53%±18, 57.8%±12) and CTL#2 (CAR and mOrange; 54%±18 and 52%±20). The modified CTL were functional, and cells transduced with either vector were able to kill the Kappa+ B cell tumor Daudi in Cr^{51} assay (72%±13 and 69%±25, respectively) at an R:S of 40:1. We confrmed the function of IL7Ra (#1) by measuring pSTAT5 and cell proliferation after IL7 administration (67,648±2703 CPM vs 8,764±793 CPM when cells were cultured in media). In addition, we were able to measure IL7 cytokine from product #2 by ELISA. IL7 production was dependent on the intensity of antigenic stimulation as demonstrated using different ratios of CTL:EBV-LCLs (4:1, 2:1, 1:1 and 1:2 which induced 3.7, 20, 99, and 192pg/ml IL7, respectively). Finally to demonstrate that product #1 could sustain #2, we mixed the cultures at a 1:4 ratio. Over 3 weeks, CTL#1 progressively increased (14% to 87%), while the frequency of CTL#2 progressively declined over the same period (63% to 5%).

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HIGH-YIELD OF CD34+ CELLS WITH BORTEZOMIB-BASED MOBILIZATION REGIMEN IS ASSOCIATED WITH SPECIFIC GENOMIC EXPRESSION PATTERNS, DECREASE IN SDF-1 PLASMA LEVELS AND UP-REGULATION OF CXCR4 IN MULTIPLE MYELOMA (MM) PATIENTS

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Typical stem cell mobilization regimens in MM include G-CSF alone or in combination with plerixafor or high-dose cyclophosphamide (CTX). Given the known *in vitro/in vivo* synergy between bortezomib (VELCADE, Vel) and alkylating agents, we investigated the potential for concurrent cytoreduction by adding Vel to the mobilization regimen. Eligible patients (pts) had symptomatic, Durie-Salmon stage II/III MM. All pts received six 21-day cycles of Vel/dexamethasone ± liposomal doxorubicin. Cycle 7 only, mobilization (Vel-mob) comprised Vel 1.3 mg/m^2 on days 1, 4, 8, and 11, CTX 3 g/m^2 on day 8, and Filgrastim 10 ug/kg (rhG-CSF) for 10 days from day 9. At data cut-off, 25 pts have been mobilized. In comparison to pts receiving just G-CSF alone, there was a significant increase in the median CD34+ collection (22.6×10^6/kg vs 10.6 10^6/kg). In addition, the number of CD34+ cells/kg collected far exceeded the study goal of 10X10^{6}cells/kg which is typical of using CTX and/or GCSF alone in

Patient	Days Required for Collection	Days to Collection	CD34+ Stem Cells (million/kg)	Stem Cells infused (X10^6/kg)	Viability (%)	Day of neutr. engraftment	Day of plt. engraftment
1	1	18	21.2	5.78	85	14	20
2	1	18	47.4	13.22	80	11	13
3	1	19	22	9.87	60	13	22
4	1	18	17.9	9.03	90	10	15
5	4	19	40.6	5.44	97	11	21
6	1	18	19.9	9.24	94	10	16
7	3	19	294.2	17.30	91	10	17
8	2	17	13.8	6.32	80	13	24
9	5	18	9.25	4.25, 2.74	80, 94	11	18
10	2	17	21.4	9.05	93	16	21
11	1	24	50.0	no transplant	no transplant		
12	2	19	66.12	12.83	85	11	11
13	1	18	30.4	7.38	93	11	11
14	2	16	43.6	10.02	92	12	14
15	1	19	51.0	12.72	87	13	11
16	1	17	15.56	5.31	93	11	13
17	1	17	6.8	6.66	92	12	13
18	1	17	31.67	9.2	95	11	20
19	1	17	7.8	7.40	92	10	11
20	1	16	43.9	11.06	98	10	21
21	1	18	23.2	no transplant	no transplant		
22	1	17	14.0	3.47	90	16	16
23	2	17	2.946	3.96	94	12	12
24	1	16	32.8	7.92	93	11	14
25	1	20	11.5	5.63	97	10	14
median	1	18	22	8.48	92	11	15